

Introduced greenhouse-invertebrates in Potsdam and Berlin with a focus on ants (Hymenoptera, Formicidae) with eight new records for Europe, Germany or the Berlin-Brandenburg region

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Abstract

Heated greenhouses are a hotspot for introduced species from warmer climates. We studied 24 greenhouses for their invertebrate fauna with a special focus on ants. Identifications were initialised by iNaturalist and its community, followed by literature, COI barcoding and consulting experts in some cases. We report 32 introduced species including eight new records at the regional, national or continental scale, four of which are ants. *Technomyrmex difficilis* and *Solenopsis texana* are recorded for the first time in Germany. *Plagiolepis alluaudi* and *Technomyrmex vitiensis* are new for Berlin and Brandenburg. *Aleptia* cf. *viatrix* (Diptera, Psychodidae) is first recorded for continental Eurasia. *Cryptotermes cavifrons* (Blattodea, Kalotermitidae) and *Geonemertes pellaensis* (Hoploneurtea, Prosorhochmidae) are recorded the first time for Europe. An unidentified species of the genus *Anisorhynchodemus* (Tricladida, Geoplanidae) is recorded the first time for Germany. Here, we present records for 37 species (five of the ant species are native), all associated with pictures in iNaturalist. For 33 individuals of 20 species, we also provide COI sequence data supporting their identification. Furthermore, the comparison of greenhouse metadata with species composition showed that the introduced ant species are dependent on a high minimum temperature in the greenhouse.

Key Words

Biological invasions, COI barcoding, greenhouse fauna, iNaturalist, introduced species, new records

Introduction

The introduction of non-native species is a side effect of globalisation and the associated international trade of goods (Westphal et al. 2008). A major new report by the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES 2023) summarises the threats posed by non-native species to the economy, food security and human health and their role in global biodiversity change. IPBES (2023) lists 37,000 species that have been introduced outside their native range. However, this process is highly dynamic, has accelerated considerably in recent years and the number of species involved may be grossly underestimated.

In Germany, 1,280 non-native species are listed, of which only 119 are insects and all have been found in the wild in outdoor habitats (<https://neobiota.bfn.de/grundlagen/anzahl-gebietsfremder-arten.html>). The German assessment largely (but not completely) ignores non-native species that have become established in anthropogenic indoor habitats, such as heated greenhouses, industrial buildings and living rooms. These indoor species may have been considered of little relevance, as they may remain confined to indoor habitats and may not be able to pose a threat to nature and the economy. Whether this will remain the case as temperatures rise and conditions change due to climate change remains a question to be investigated in the future.

To create the (sub)tropical environment typical of heated greenhouses, soil and plant material have been imported from a wide range of tropical and subtropical countries and plants have also been exchanged amongst greenhouses. This results in a high potential for species not only to be introduced, but also to spread and to become established over time. The stable climatic conditions allow tropical species to build up populations and become abundant in greenhouses (Blatrix et al. 2018), creating completely new ecosystems (Hobbs et al. 2009). These species can become pests that are difficult and costly to control (Kenis and Branco 2010; Blatrix et al. 2018) and their establishment carries the risk that they, or their diseases, can spread from the greenhouses to outdoor habitats. By this, greenhouses can act as global ‘disease pools’ infecting other greenhouses with alien species. It is also interesting to investigate whether there is a common set of global species that occur in greenhouses and form their own ecological communities. Invertebrates, in particular, can be rapidly introduced into greenhouses and often remain undetected for long periods due to their often small size and hidden lifestyle (Stohlgren and Schnase 2006; Mehta et al. 2007). Furthermore, they are difficult to identify as identification resources often just cover national faunas, rarely including exotic species, are restricted to outdoor ecosystems or are too old to include recently arrived species (Suvák 2011; Blatrix et al. 2018; Báthori et al. 2024).

The aim of this study is to document non-native invertebrates in heated greenhouses in Berlin and Potsdam, with a particular focus on ants. Ants are amongst the most successful and damaging invaders worldwide (Lowe et al. 2000). The Global Invasive Species Database contains 19 invasive ant species and five ant species that are amongst the 100 worst invasive species (GISD 2024). So-called tramp species, which have been spread globally by human activities, can arise. The high adaptability of ants to new habitats is often due to social traits, such as polygyny, polydomy and super-colonial colony structure (Tsutsui and Suarez 2003). Tramp species in greenhouses are not able to colonise outdoors due to current climatic conditions (Boer and Vierbergen 2008). However, such tramp species are often species that are invasive in warmer climates. For example, nearly all tramp species found in this study are classified as “highly invasive” in AntWiki (<https://www.antwiki.org>), which means that they are “species that are essentially or actually worldwide in distribution” (<https://www.antwiki.org>). The only found tramp species that does not have an active AntWiki page is also a cosmopolitan tramp species (Seifert 2013). Ants in greenhouses can sometimes be very dominant and indirectly damage plants through their symbiotic relationship with hemipterans (Blatrix et al. 2018). For these reasons, their study is particularly important and ants have been collected more intensively. However, little is known about introduced invertebrate species in greenhouses in Germany and especially the occurrence of tramp ants is poorly understood. This

study aims to better assess the diversity of introduced invertebrates in greenhouses in Berlin and Potsdam. In addition, the occurrence of ant species in combination with temperature in greenhouses is assessed to better understand their lower temperature limits. This is an important factor for the spread and distribution of species. Furthermore, we aim to test whether a combination of morphological identification by AI and experts as well as genetic identification allows us to properly identify species that come from anywhere in the world.

Methods

The study examined six facilities (Fig. 1) in Berlin and Potsdam. These were divided into 24 climatic units. A climatic unit is a contiguous greenhouse or contiguous greenhouse section where the same climatic conditions prevail. For example, the Berlin Botanical Garden has many greenhouse sections that are heated to different degrees or have different air humidity. Therefore, 14 climatic units (Table 1) are distinguished. The metadata for each climatic unit (size of the greenhouse in m², use of the greenhouse, minimum temperature in °C, average humidity in %, use of insecticides or biological pest control, when the greenhouse was surveyed and the exact location of the greenhouse) are given in Table 1. These metadata were compared with the ant species composition.

The Pearson-correlation analysis was carried out in Excel (2024) to find out whether the number of introduced and native ant species and those of native species correlate with the minimum temperatures of the greenhouses.

Each greenhouse was searched manually for invertebrates. Loose objects, such as wood and stones, were turned over and the soil underneath was inspected. We also scanned the lower foliage. Ants were additionally lured with a bait of sugar-water or a mixture of fish oil, rum and honey (as recommended by Bernhard Seifert, pers. com.). The baits were placed on small (25 × 25 mm) plastic plates and placed approximately every four metres along the walkways throughout the greenhouses. After 30–60 minutes, the baits were checked for ants. Table 2 lists all ant species found in the greenhouses.

All species were photographed (Nikon D600 with macro lens, distance rings and tong flash) and a few individuals of each species were transferred to 90% ethanol. Georeferenced records were uploaded to the global reporting platform iNaturalist (www.inaturalist.org). All records are available on the iNaturalist profiles eliasfreyhof36614’s Profile iNaturalist and emilvus’s Profile iNaturalist. iNaturalist’s artificial intelligence (computer vision) suggested an initial taxonomic identification, which was then critically reviewed by us. The iNaturalist community either suggested alternative species identifications or confirmed ours, a process that was an additional step towards a valid identification. These identifications were made by both amateur and expert naturalists. At least two matching identifications or two-thirds of matching

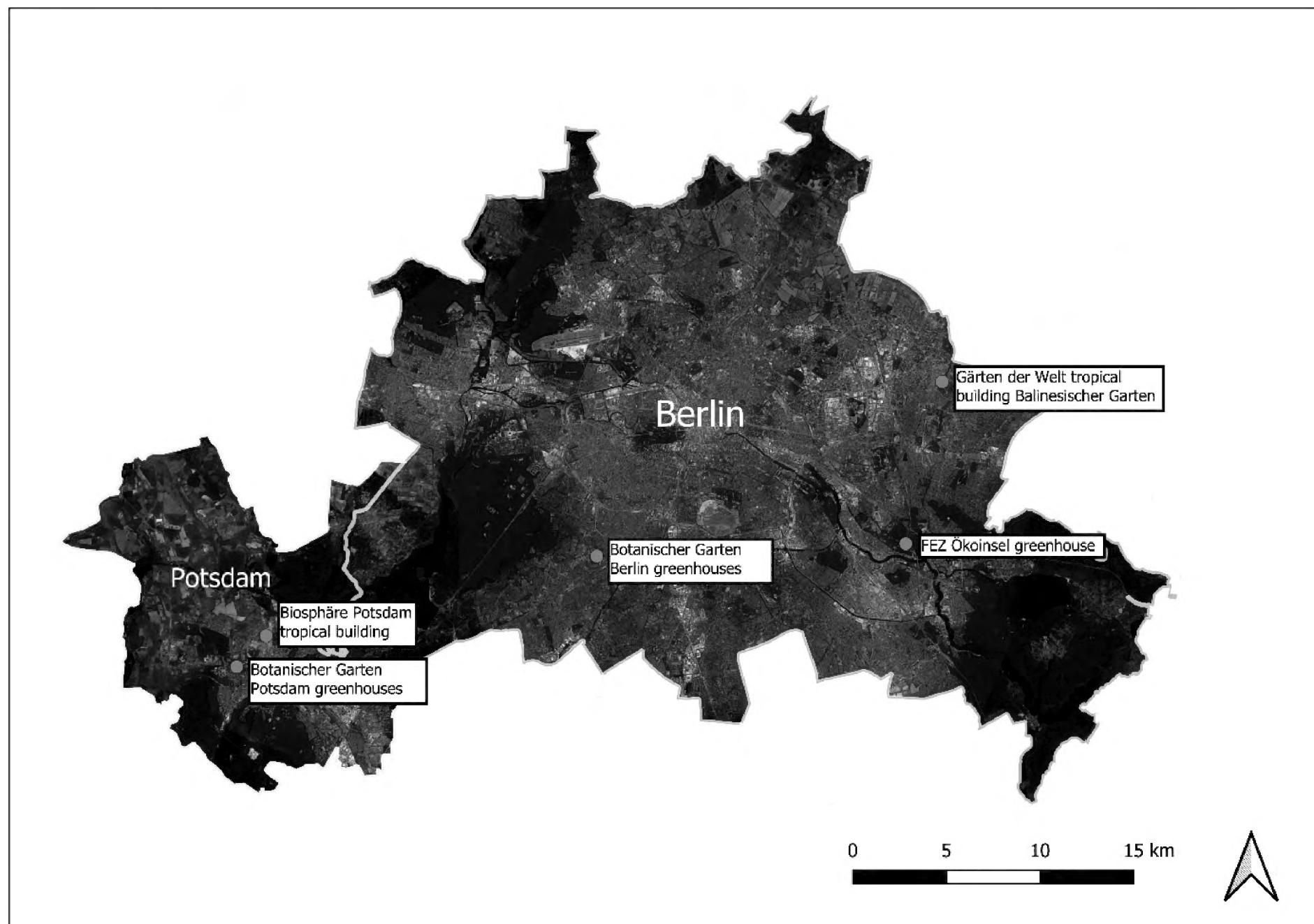


Figure 1. Location of greenhouses studied in Berlin and Potsdam. © GeoBasis-DE/LGB (2024), dl-de/by-2-0

identifications were required to achieve ‘research grade’ at iNaturalist. Some species that were difficult to identify from a photograph were identified morphologically and/or genetically. Morphology was examined using binoculars and specialist literature (Table 3). Species identification remained a challenge due to the introduction of species from all over the world and the fact that literature is usually based on specific regions. Accordingly, a search was made to determine which species of a genus had already been introduced elsewhere. Suitable identification keys or publications were then searched for the potentially introduced species. Ants of the genera *Technomyrmex* and *Solenopsis* were sent to Bernhard Seifert (Görlitz) for morphometric identification. Diplopoda, Gastropoda, Hoplonemertea and Turbellaria were re-determined by experts on the basis of photographs (Table 3). The collected specimens are deposited at the Senckenberg German Entomological Institute, Müncheberg (Germany). *Solenopsis* spp., *Technomyrmex* spp., *Oxidus gracilis*, *Leptogenius sororinus* and *Stenochrus portoricensis* are deposited in the collection of the Senckenberg Museum für Naturkunde Görlitz (Germany).

DNA extraction and PCR

DNA was extracted from whole samples, leg or femur muscle tissue (stored in ethanol) using sterilised tweezers.

After allowing the ethanol to evaporate, DNA was extracted using a Chelex extraction protocol (modified from Walsh et al. 1991). For each sample, 100 µl Chelex solution (5%) and 5 µl proteinase K (10 mg/ml) were added. All samples were then placed in a thermocycler (Labcycler Basic, SensoQuest, Göttingen, Germany) with the following program: 1 h at 55 °C, 15 min at 99 °C, 1 min at 37 °C. Extracts were stored at -20 °C.

The barcoding fragment of the mitochondrial COI gene was amplified using Qiagen DNA polymerase (QIAGEN Multiplex PCR Plus Kit, Qiagen, Hilden, Germany) at a concentration of 50% of the total reaction volume and a concentration of 33% of each primer. Primers LCO_1490 and HCO_2198 (Folmer et al. 1994) were used for the first attempt. Primers CLepFol R / CLepFol F, LCO1490-JJ2 / HCO2198-JJ2 (Astrin et al. 2016) and LCO1490-JJ / LCO1490-JJ (Astrin and Stüben 2008) were used for samples that did not work previously. The PCR programme included 38 cycles (5 min at 95 °C, 38 × (30 s at 95 °C, 90 s at 49 °C, 60 s at 72 °C), 30 min at 68 °C). The PCR products were then checked on a 1.3% agarose gel. Positive samples were purified using the ExoCleanUp FAST Kit (VWR International GmbH, Darmstadt, Germany) and sent to Macrogen Europe B.V. (Amsterdam, The Netherlands) for Sanger sequencing. Sequences were edited using Geneious (version R11) and then uploaded to BOLD Systems (v.4) (<https://www.boldsystems.org/>) under project ID GBAR (Table 4).

Table 1. Climatic units (see Material and methods for abbreviations).

Greenhouse	Size in m ²	Usage	Minimum temperature in °C	Average air humidity in %	Insecticides or biological pest control use	Date of visit	Coordinates	Abbreviation of climatic units
Gärten der Welt (GW.), Balinese Garden ("Balinesischer Garten")	1200	plant cultivation	18	80	Biological pest control: Ichneumonidae wasps two times a month	13.11.2023	52°32.37'N, 13°34.656'E	/
FEZ., Eco Island ("Ökoinsel")	300	plant cultivation	10	45	Insecticides: Ant bait boxes if needed Neudorff Loxiran https://www.neudorff.de/produkte/loxiran-ameisenkoederdose.html	24.11.2023	52°27.7533'N, 13°33.075'E	/
Botanical Garden Berlin (BGB.), house A	1900	plant cultivation	19	55	Biological pest control: Cryptolaemus montrouzieri, Anolis sagrei, Ichneumonidae, Eleutherodactylus coqui and more Insecticides: In winter, individual plants are sprayed with insecticides. If there are too many ants, they are controlled with gels.	22.11.2023	52°27.395'N, 13°18.447'E	/
BGB., house B	170	plant cultivation	18	70	as BGB.	27.11.2023, 14.05.2024	52°27.375'N, 13°18.467'E	B
BGB., house C	500	plant cultivation	17	70	as BGB.	27.11.2023, 14.05.2024	52°27.365'N, 13°18.478'E	C
BGB., house D	135	plant cultivation	15	70	as BGB.	27.11.2023, 14.05.2024	52°27.357'N, 13°18.462'E	D
BGB., house E	200	plant cultivation	17	58	as BGB.	27.11.2023, 14.05.2024	52°27.350'N, 13°18.445'E	E
BGB., house F	370	plant cultivation	17	70	as BGB.	27.11.2023, 14.05.2024	52°27.358'N, 13°18.437'E	F
BGB., house G	200	plant cultivation	17	50	as BGB.	27.11.2023, 14.05.2024	52°27.365'N, 13°18.425'E	G
BGB., house H/I	570	plant cultivation	12	45	as BGB.	29.12.2023, 14.05.2024	52°27.395'N, 13°18.395'E	H/I
BGB., house K	200	plant cultivation	6	60	as BGB.	29.12.2023, 14.05.2024	52°27.408'N, 13°18.378'E	K
BGB., house L	135	plant cultivation	10	50	as BGB.	29.12.2023, 14.05.2024	52°27.413'N, 13°18.395'E	L
BGB., house M	500	plant cultivation	10	45	as BGB.	29.12.2023, 14.05.2024	52°27.422'N, 13°18.413'E	M
BGB., house N	170	plant cultivation	8	60	as BGB.	29.12.2023, 14.05.2024	52°27.413'N, 13°18.422'E	N
BGB. Cultivation house GW.-02-Blü	275	plant cultivation	19	50	as BGB.	09.01.2024	52°27.315'N, 13°18.485'E	O
BGB. cultivation house 52°27.147'N, 13°18.677'E	220	plant cultivation	18	60	as BGB.	09.01.2024	52°27.147'N, 13°18.676'E	P
Biosphere Potsdam (BP.)	5000	show greenhouse	19	94	Biological pest control: Cryptolaemus montrouzieri, Ichneumonidae, Nematoda, Chalcidoidea Insecticides: Neudorff Loxiran Ameisenköderdosen: https://www.neudorff.de/produkte/loxiran-ameisenkoederdose.html https://www.unkrautvernichter-shop.de/IMIDASECT-Schabengel.html https://www.unkrautvernichter-shop.de/imidasect-ants.html	16.01.2024, 17.01.24	52°25.112'N, 13°02.917'E	/
Botanical Garden Potsdam (BGP.), Palmenhaus	300	plant cultivation	14	60	Biological pest control: multiple species Insecticides: Spruzit Pyritrin	30.01.2024	52°24.219'N, 13°01.563'E	PH
BGP., Epiphytenhaus	220	plant cultivation	19	100	as BGP.	31.01.2024	52°24.219'N, 13°01.563'E	EH
BGP. Nutzpflanzenhaus	225	plant cultivation	18	70	as BGP.	01.02.2024	52°24.213'N, 13°01.540'E	NH
BGP., Haus der tropischen Vielfalt	225	plant cultivation	18	80	as BGP.	02.02.2024	52°24.215'N, 13°01.527'E	HTV
BGP., Kakteenhaus	190	plant cultivation	10	50	as BGP.	03.02.2024	52°24.215'N, 13°01.515'E	KH
BGP., Victoriahaus	315	plant cultivation	19	80	as BGP.	04.02.2024	52°24.227'N, 13°01.533'E	VH
BGP., Farnhaus	220	plant cultivation	16	80	as BGP.	05.02.2024	52°24'13.5"N, 13°01'33.2"E	FH

Table 2. Ant records (see Material and methods for abbreviations).

Species	Recorded from	New record(s)	Native range	Invasiveness (ranked by antwiki.org)	Identification based on
<i>Lasius emarginatus</i> (Olivier, 1792)	BGB. (A, E, H/I, K, L, M, N), BGP. (VH, FH)	/	Native	/	Seifert (2018), barcoding, iNat RG, iNat AI
<i>Lasius niger</i> (Linnaeus, 1758)	BGB. (A, E, H/I, K, L, M, N), BGP. (PH, FH), FEZ.	/	Native	/	Seifert (2018), iNat AI
<i>Ponera coarctata</i> (Latreille, 1802)	BGB. (K)	/	Native	/	Seifert (2018), barcoding, iNat RG, iNat AI
<i>Solenopsis fugax</i> (Latreille, 1798)	BGP. (PH)	/	Native	/	Seifert (pers. com.), barcoding, Seifert (2018), iNat RG, iNat AI
<i>Tetramorium caespitum</i> complex	BGP. (PH, KH), FEZ.	/	Native	/	Seifert (2018), iNat RG, iNat AI
<i>Hypoponera ergatandria</i> (Forel, 1893)	BGP. (PH, VH), BP.	/	unknown	highly invasive (cosmopolitan)	Seifert (2018), barcoding
<i>Plagiolepis alluaudi</i> Emery, 1894	BGB. (A, B, C, D, E, F, G, O, P), BGP. (EH)	Berlin & Brandenburg	Africa (Wetterer 2014)	highly invasive (cosmopolitan)	Wetterer (2014), barcoding, iNat RG, iNat AI
<i>Solenopsis texana</i> Emery 1895	BP., GW.	Germany	the americans		Seifert (pers. com.), barcoding
<i>Technomyrmex difficilis</i> Forel, 1892	BP.	Germany	Madagascan region (Wetterer 2013)	highly invasive (cosmopolitan)	Seifert (pers. com.), Bolton (2007), barcoding, iNat RG
<i>Technomyrmex vitiensis</i> Mann, 1921	BGB. (A, B, C, D, O), BGP. (PH, EH, NH, HTV, VH, FH)	Berlin & Brandenburg	Southeast Asian region (Bolton 2007)	highly invasive (cosmopolitan)	Seifert (pers. com.), Bolton (2007), barcoding, iNat RG
<i>Tetramorium bicarinatum</i> (Nylander, 1846)	BGB. (A, B, C)	/	Oriental region (Wetterer 2009)	highly invasive (cosmopolitan)	Garcia and Fisher (2011), barcoding, iNat RG, iNat AI

Abbreviations

BGB: Botanical Garden Berlin, **BGP:** Botanical Garden Potsdam, **BP:** Biosphere Potsdam, **GW:** Gärten der Welt, **FEZ:** FEZ. Ökoinsel greenhouse.

Results

A total of 32 introduced species were found in the five institutions. Insects are the largest taxonomic group with 14 species, including six species of ants, one species of termites, two cockroaches, two Orthoptera, one earwig, one weevil and one drainfly species. Seven species belong to spiders and, except for one species of *Schizomyida*, all belong to the order Araneae. There were also six species of snails, two species of millipedes, one species of isopods, one species of amphipods, one species of terrestrial planaria and one species of terrestrial nemertine (Tables 2, 3).

From these, we report eight species for the first time at the regional, national or continental scale, i.e. we found no published or unpublished records:

- *Technomyrmex difficilis* (Hymenoptera, Formicidae) is reported the first time for Germany.
- One of two *Solenopsis* (Hymenoptera, Formicidae) species found by us should be *Solenopsis texana*. This species is recorded for the first time from Germany.
- *Plagiolepis alluaudi* (Hymenoptera, Formicidae) is new for Berlin and Brandenburg.
- *Technomyrmex vitiensis* (Hymenoptera, Formicidae) is new for Berlin and Brandenburg.
- *Aleptia cf. viatrix* (Diptera, Psychodidae) is a new record for continental Eurasia.

- *Cryptotermes cavifrons* (Blattodea, Kalotermitidae) is new for Europe.
- *Geonemertes pelaensis* (Hoploneurida, Prosorhachmidae) is new for Europe.
- One so-far unidentified species of the genus *Anisorrhynchodemus* (Trichladida, Geoplanidae) is first recorded for Germany.

iNaturalist (based on AI and/or community identifications) correctly identified 31 out of a total of 37 native and non-native species (83.8%). Of the eight new records, iNaturalist was able to identify five species. Twenty of the species were successfully barcoded. The attempt to barcode three additional species failed and the rest of the species were only photographed. In 13 cases, specialist literature was used for morphological identification. Of the total 37 species, 14 species were identified by experts (Tables 2, 3).

Correlation of minimal greenhouse temperatures with ant species numbers supports that the number of introduced species is dependent on high minimum temperatures, with the Pearson-correlation being significantly positive ($r = 0.856$; $p < 0.01$). For the native species, the correlation was negative, but not significant ($r = -0.581$; $p = 0.078$). Thus, native species occurred at almost all minimum temperatures (nine of the ten possible), while introduced species were not found in climatic units with a minimum temperature of 13 °C or less (Fig. 2). This temperature dependence was observed quite well in the BGB. The ants can move between climatic units there because the climatic units are not physically isolated. In climatic unit A, the minimum temperature was the highest with 19 °C. *Tetramorium bicarinatum*, *Technomyrmex vitiensis* and *Plagiolepis alluaudi* were abundant. In the adjoining two

Table 3. Other introduced greenhouse fauna (see Material and methods for abbreviations).

Class	Order	Species	Recorded for	New record(s)	Native range	Identification based on
Insecta	Orthoptera	<i>Gryllodes sigillatus/supplicans</i>	BGB.	/	South-western Asia (www.speciesfile.org 2024)	barcoding, iNat RG, iNat AI
Insecta	Orthoptera	<i>Tachycines asynamorus</i> (Adelung, 1902)	FEZ., GW.	/	East Asia (Boettger 1950)	barcoding, iNat RG, iNat AI
Insecta	Blattodea	<i>Cryptotermes cavifrons</i> Banks, 1906	BP.	Europe	Florida (Brammer and Scheffrahn 2002)	barcoding, Scheffrahn and Krecek (1999); Scheffrahn and Su (1994)
Insecta	Blattodea	<i>Periplaneta australasiae</i> (Fabricius, 1775)	BGB., BGP., BP.	/	Africa (Rehn 1945)	barcoding, iNat RG, iNat AI
Insecta	Blattodea	<i>Pycnoscelus surinamensis</i> (Linnaeus, 1758)	BP.	/	Indo-Malaysia (Gade and Parker 1997)	barcoding, iNat RG, iNat AI
Insecta	Dermoptera	<i>Euborellia arcanum</i> Matzke & Kočárek, 2015	BP.	/	unknown	barcoding, iNat RG, iNat AI
Insecta	Coleoptera	<i>Sitophilus oryzae</i> A.Hustache, 1930	BP.	/	likely Indian subcontinent (Corrêa et al. 2017)	barcoding, iNat AI
Insecta	Diptera	<i>Aleptia cf.viatrix</i> Jaume-Schinkel, Kvifte, Weele & Mengual, 2022	BGP.	Continental Eurasia	Neotropics (Jaume- Schinkel et al. 2023)	barcoding
Arachnida	Araneae	<i>Amaurobius ferox</i> (Walckenaer, 1830)	BGB.	/	Mediterranean region (Bellmann 2001)	iNat RG, iNat AI
Arachnida	Araneae	<i>Hasarius adansoni</i> (Audouin, 1826)	BGB., BGP., BP., GW.	/	Africa (World Spider Catalogue 2017)	iNat RG, iNat AI
Arachnida	Araneae	<i>Holocnemus pluchei</i> (Scopoli, 1763)	BGP.	/	Mediterranean region (World Spider Catalogue 2016)	iNat RG
Arachnida	Araneae	<i>Parasteatoda tepidariorum</i> (C.L.Koch, 1841)	BGB., BGP., BP.	/	unknown	iNat RG, iNat AI
Arachnida	Araneae	<i>Pholcus phalangioides</i> (Fuesslin, 1775)	BP.	/	Western Asia (World Spider Catalog 2024)	iNat RG, iNat AI
Arachnida	Araneae	<i>Uloborus plumipes</i> Lucas, 1846	BGP., BP.	/	Old World (World Spider Catalog 2020)	iNat RG, iNat AI
Arachnida	Schizomida	<i>Stenochrus portoricensis</i> Chamberlin, 1922	BP.	/	Southeast Asia (Rowland and Reddell 1977)	barcoding, Lauterbach et al. (2020)
Diplopoda	Spirobolida	<i>Leptogoniulus sorormus</i> (Butler, 1876)	BP.	/	Southeast Asia (Decker et al. 2014)	Peter Decker (pers. com.), iNat RG, iNat AI
Diplopoda	Polydesmida	<i>Oxidus gracilis</i> (C.L.Koch, 1847)	BGB., BGP., BP.	/	East Asia (Decker et al. 2014)	barcoding, iNat RG, iNat AI
Malacostraca	Amphipoda	<i>Talitroidea</i>	BGP.	/		iNat: one expert opinion
Malacostraca	Isopoda	<i>Porcellio dilatatus</i> Brandt, 1831	BGP.	/	Southwest Europe (Allspach 1992)	Andreas Allspach (pers. com.), iNat RG, iNat AI
Gastropoda	Stylommatophora	<i>Ambigolimax valentianus</i> (A.Férussac, 1821)	BGB., BGP.	/	Southwest Europe (www.naturportal- suedwest.de 2024)	Carsten Renker (pers. com.), iNat RG, iNat AI
Gastropoda	Stylommatophora	<i>Hygromia cinctella</i> (Draparnaud, 1801)	BGB.	/	Italy and surrounding regions (Beckmann and Kobialka 2008)	Carsten Renker (pers. com.), iNat RG, iNat AI
Gastropoda	Sorbeboconcha	<i>Melanoides tuberculata</i> (O.F.Müller, 1774)	BP.	/	unknown	Carsten Renker (pers. com.), iNat RG, iNat AI
Gastropoda	Pulmonata	<i>Physella acuta</i> (Draparnaud, 1805)	BGP.	/	North America (Semenchenko et al. 2008)	Carsten Renker (pers. com.), iNat RG, iNat AI
Gastropoda	Stylommatophora	<i>Subulina cf. octona</i> (Bruguière, 1789)	BGP., BP.	/	Caribbean (Deisler and Abbott 1984)	Carsten Renker (pers. com.)
Hoploneurida	Monostilifera	<i>Geonemertes pelaensis</i> Semper, 1863	BP.	Europe	Indopacific region (Morffe et al. 2020)	Leigh Winsor (pers. com.), barcoding, iNat RG, iNat AI
Turbellaria	Tricladida	<i>Anisorhynchodemus</i> sp.	BP.	Germany	unknown	Leigh Winsor (pers. com.), iNat RG, iNat AI

climatic units, B and C, with minimum temperatures of 18 and 17 °C, respectively, the species composition did not change at all. At 17 °C minimum, *Technomyrmex vitiensis* was more dominant than *Tetramorium bicarinatum*. In the following climatic unit D with 15

°C, *Tetramorium bicarinatum* was missing. The other two species were also much less abundant than in the warmer climatic units. In climatic unit H/I with a minimum temperature of 12 °C, none of the introduced species was found (Fig. 2).

Table 4. Genetic data of collected specimens.

Species	Sample ID	Collection Date	Recorded from	Coordinates (Lat, Lon)	Bold ID	Highest similarity	Possible species COI
<i>Cryptotermes cavifrons</i>	DEI-Hemimetabola100424	16.01.2024	BP.	52.4185, 13.0486	GBAR001-24	99.85% to TMTER440-15 (<i>Cryptotermes cavifrons</i>)	/
<i>Tetramorium bicarinatum</i>	DEI-GISHym5391	22.11.2023	BGB.	52.4564, 13.3076	GBAR002-24	100% to ASANA722-06 (<i>Tetramorium bicarinatum</i>)	/
<i>Tetramorium bicarinatum</i>	DEI-GISHym5392	22.11.2023	BGB.	52.4564, 13.3076	GBAR003-24	100% to ASANA722-06 (<i>Tetramorium bicarinatum</i>)	/
<i>Stenochrus portoricensis</i>	SMNG_24/67178/1	17.01.2024	BP.	52.4185, 13.0486	GBAR004-24	100% to GACO1420-19 (<i>Stenochrus portoricensis</i>)	/
<i>Stenochrus portoricensis</i>	SMNG_24/67178/2	17.01.2024	BP.	52.4185, 13.0486	GBAR005-24	100% to GACO1420-19 (<i>Stenochrus portoricensis</i>)	/
<i>Technomyrmex difficilis</i>	DEI-GISHym5393	16.01.2024	BP.	52.4185, 13.0486	GBAR006-24	100% to ASANA512-06 (<i>Technomyrmex difficilis</i>)	/
<i>Sitophilus oryzae</i>	SDEI-Coleoptera304561	16.01.2024	BP.	52.4185, 13.0486	GBAR007-24	100% to CICRP053-15 (<i>Sitophilus oryzae</i>)	/
<i>Technomyrmex vitiensis</i>	DEI-GISHym5394	22.11.2023	BGB.	52.4564, 13.3076	GBAR008-24	100% to LEFIJ12092-20 (<i>Technomyrmex vitiensis</i>)	/
<i>Technomyrmex vitiensis</i>	DEI-GISHym5395	22.11.2023	BGB.	52.4564, 13.3076	GBAR009-24	100% to LEFIJ12092-20 (<i>Technomyrmex vitiensis</i>)	/
<i>Plagiolepis alluaudi</i>	DEI-GISHym5396	21.11.2023	BGB.	52.4564, 13.3076	GBAR010-24	99.84% to ASAMZ391-07 (<i>Plagiolepis alluaudi</i>)	<i>P. alluaudi</i> , <i>Nylanderia madagascarensis</i>
<i>Hypoponera ergatandria</i>	DEI-GISHym5397	30.01.2024	BGP.	52.4038, 13.0254	GBAR011-24	100% to NODRY624-15 (<i>Hypoponera ergatandria</i>)	<i>H. punctatissima</i> , <i>H. ergatandria</i>
<i>Hypoponera ergatandria</i>	DEI-GISHym5398	30.01.2024	BGP.	52.4038, 13.0254	GBAR012-24	100% to NODRY624-15 (<i>Hypoponera ergatandria</i>)	<i>H. punctatissima</i> , <i>H. ergatandria</i>
<i>Ponera coarctata</i>	DEI-GISHym5399	29.12.2023	BGB.	52.4568, 13.3063	GBAR013-24	100% to ANTBG104-11 (<i>Ponera coarctata</i>)	<i>P. coarctata</i> , <i>P. testacea</i>
<i>Lasius emarginatus</i>	DEI-GISHym5400	30.01.2024	BGP.	52.4037, 13.0255	GBAR014-24	100% to NOANT006-12 (<i>Lasius niger</i>)	<i>L. niger</i> , <i>L. platythorax</i> , <i>L. brunneus</i> , <i>L. paralienus</i> , <i>L. emarginatus</i>
<i>Lasius emarginatus</i>	DEI-GISHym5401	30.01.2024	BGP.	52.4037, 13.0255	GBAR015-24	100% to NOANT006-12 (<i>Lasius niger</i>)	<i>L. niger</i> , <i>L. platythorax</i> , <i>L. brunneus</i> , <i>L. paralienus</i> , <i>L. emarginatus</i>
<i>Tachycines asynamorus</i>	DEI-Hemimetabola100425	14.11.2023	GW.	52.5395, 13.5777	GBAR016-24	100% to GBORT531-14 (<i>Tachycines asynamorus</i>)	/
<i>Periplaneta australasiae</i>	DEI-Hemimetabola100426	22.11.2023	BGB.	52.4564, 13.3076	GBAR017-24	100% to VAQT477-09 (<i>Periplaneta australasiae</i>)	/
<i>Pycnoscelus surinamensis</i>	Hemimetabola100427	16.01.2024	BP.	52.4185, 13.0486	GBAR018-24	100% to TTSOW223-10 (<i>Pycnoscelus surinamensis</i>)	/
<i>Euborellia arcum</i>	DEI-Hemimetabola100428	17.01.2023	BP.	52.4185, 13.0486	GBAR019-24	100% to GBMND6588-21 (<i>Euborellia arcum</i>)	/
<i>Gryllodes sigillatus/ supplicans</i>	DEI-Hemimetabola100429	14.05.2024	BGB.	52.4565, 13.3066	GBAR020-24	99.08% to MAORT859-12 (<i>Gryllodes supplicans</i>)	<i>G. sigillatus</i> , <i>G. supplicans</i>
<i>Oxidus gracilis</i>	VNR_021924	29.12.2023	BGB.	52.4568, 13.3063	GBAR021-24	100% to MYRUS048-09 (<i>Oxidus gracilis</i>)	/
<i>Geonomertes pelaensis</i>	DEI-Hemimetabola100430	16.01.2024	BP.	52.4185, 13.0486	GBAR022-24	100% to GBSP16798-19 (<i>Geonomertes pelaensis</i>)	/
<i>Plagiolepis alluaudi</i>	DEI-GISHym5402	09.01.2024	BGB.	52.4524, 13.3113	GBAR023-24	100% to ASAMZ391-07 (<i>Plagiolepis alluaudi</i>)	<i>P. alluaudi</i> , <i>Nylanderia madagascarensis</i>
<i>Alepia cf. viatrix</i>	DEI-Hemimetabola100431	30.01.2024	BGP.	52.4035, 13.0256	GBAR024-24	100% to ASMII2842-22 (<i>Alepia viatrix</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5403	16.01.2024	BP.	52.4185, 13.0486	GBAR025-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5404	16.01.2024	BP.	52.4185, 13.0486	GBAR026-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5405	16.01.2024	BP.	52.4185, 13.0486	GBAR027-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5406	14.11.2023	GW.	52.5395, 13.5777	GBAR028-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5407	14.11.2023	GW.	52.5395, 13.5777	GBAR029-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Solenopsis texana</i>	DEI-GISHym5408	14.11.2023	GW.	52.5395, 13.5777	GBAR030-24	100% to ASPNA1425-10 (<i>Solenopsis saudiensis</i>)	/
<i>Technomyrmex difficilis</i>	DEI-GISHym5409	16.01.2024	BP.	52.4185, 13.0486	GBAR031-24	100% to ASANA512-06 (<i>Technomyrmex difficilis</i>)	/
<i>Solenopsis fugax</i>	DEI-GISHym5410	30.01.2024	BGP.	52.4036, 13.0261	GBAR032-24	100% to ANTBG284-11 (<i>Solenopsis fugax</i>)	/
<i>Technomyrmex vitiensis</i>	DEI-GISHym5411	30.01.2024	BGP.	52.4036, 13.0261	GBAR033-24	100% to LEFIJ12092-20 (<i>Technomyrmex vitiensis</i>)	/
<i>Tetramorium caespitum complex</i>	DEI-GISHym5412	22.11.2023	BGB.	52.4036, 13.0261	/	/	/
<i>Lasius niger</i>	DEI-GISHym5413	22.11.2023	BGB.	52.4564, 13.3076	/	/	/
<i>Leptogeniulus sororius</i>	VNR_021982	17.01.2024	BP.	52.4185, 13.0486	/	/	/

Discussion

We found no introduced ant species in climatic units with minimum temperatures lower than 14 °C and the number of introduced species correlates positively with higher minimum temperatures. Our study shows that minimum temperature limits the colonisation success of introduced species (Fig. 2). This dataset could be useful for modelling the potential distribution of invasive ant species under future climate change conditions. Furthermore, minimising temperatures, where possible, could be a possible measure for controlling introduced ants in greenhouses. However, further research is needed in this field.

iNaturalist provides an efficient, global method of species identification, with its AI trained on millions of images and currently covering 93,369 species. It excels at identifying common species with distinctive characters, but struggles with cryptic species such as ants. Community identifications are a good complement to AI because many biologists and professionals, together with amateur naturalists, can make a solid identification, if good photographs with the relevant features are provided. iNaturalist also connected us to international experts who helped in identifications allowing us to identify several taxa. For example, we got to know the experts Sean Birk, Bek Craig and Leigh Winsor through iNaturalist. However, taxonomic groups with small specialist communities often go unidentified. We faced this challenge with snails. While iNaturalist is a good starting point, it was not the only method used for difficult-to-identify species.

Where samples were collected, identification was possible through literature and/or COI barcoding. Both methods

require special materials, specific knowledge and more time. However, these methods can provide very accurate identifications in many cases. Morphology-based literature requires matching keys or descriptions, which can be difficult to find for introduced species that can come from anywhere in the world. COI barcoding is more universal, but has the problem that not every individual can be identified to species level. For some species, there are few or no reference sequences in the database, which can lead to errors. At least one of these two methods was preferred by us to obtain a reliable identification of the more difficult-to-identify species.

The next method used was expert identification, which was an additional step, if one of the other methods did not work, was not possible or was not accurate enough. This method can provide a very reliable identification without a lot of knowledge and material. However, it requires contact with experts and some time. We also asked experts when only photos were taken and iNaturalist did not provide reliable identifications.

The next part of the discussion deals with the species which, as far as we could find out, are new records at the regional or even continental scale (Tables 2, 3).

Solenopsis texana (Fig. 3.)

This species of thief ant is extremely small (about 1.2 mm) and golden yellow in colour. Its native range should be the Americas, where it has been found in Canada, the USA, Mexico, Nicaragua, Costa Rica, Panama, Colombia and Brazil. It has been introduced into greenhouses in Europe,

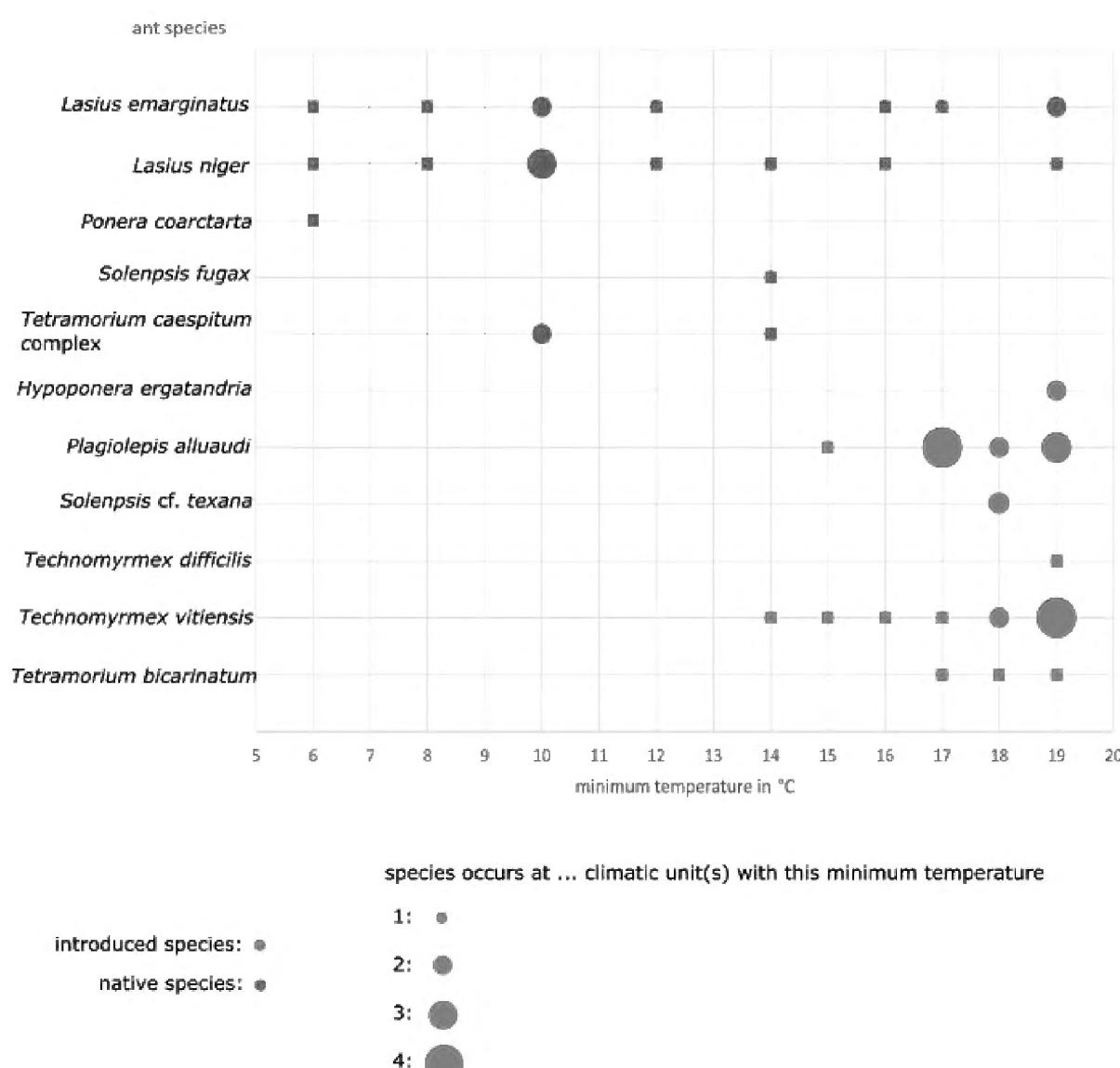


Figure 2. Records of native and non-native ant species in greenhouses with different minimum temperatures.

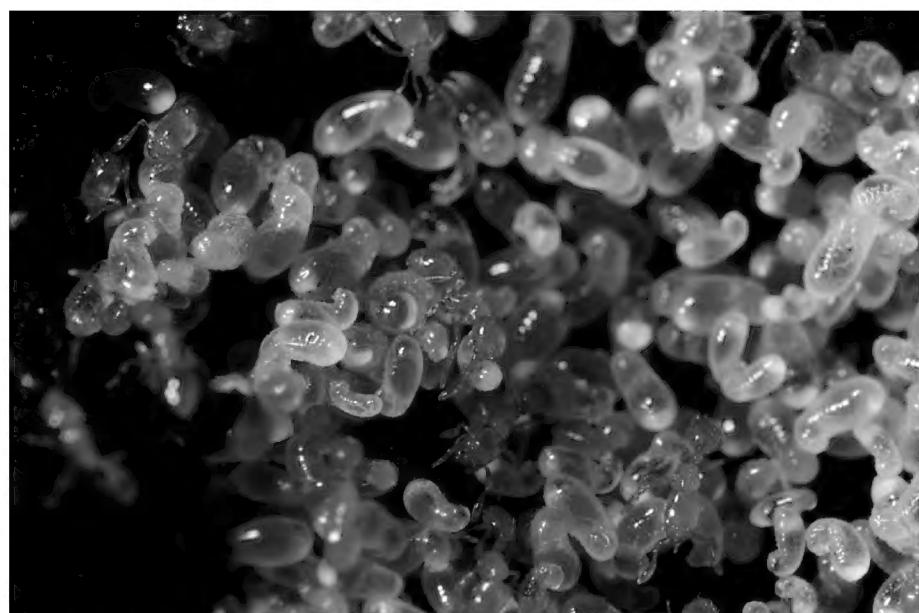


Figure 3. *Solenopsis texana* nest with workers and larvae: Gärten der Welt.

including Norway and Germany (<https://www.antwiki.org>; (GBIF 2023a); www.gbif.org; Seifert, pers. com.). Its hidden lifestyle in the soil and its small size makes it very difficult to detect. The distribution of this species is, therefore, probably underestimated. According to evaluation of the image of a worker type (specimen identifier CASENT0904625 in www.antbase.org), this *Solenopsis* species should be *Solenopsis texana* Emery 1895 (B. Seifert, pers. comm. 11 October 2024). To our knowledge, *Solenopsis texana* has never been found in Germany. The taxonomy of this species is unclear and needs to be revised (Seifert, pers. com.). Barcoding resulted in *Solenopsis saudiensis* (100% similarity on best matches), which is a junior synonym of *S. abdita* (Sharaf et al. 2020). However, not all species of the *S. molesta* complex are available in GenBank making it impossible to identify our materials only by COI barcode. Seifert (pers. com.) examined the morphometry of our specimens and found them to be conspecific with an *Solenopsis texana* found by Gjershaug et al. (2016) in a greenhouse in Norway (NOR: 58.82005°N, 9.07145°E, Kristiansand, 80 m, Dyreparken, in Tropenhaus, A. Staverlokk 2016.04.22).

Technomyrmex difficilis (Fig. 4)

This species is a 3.5–4.0 mm long, black ant native to the Madagascar region (Wetterer 2013). It has been introduced to Central and North America, where it is known as an indoor species in the north. In Europe, *T. difficilis* has been recorded for indoor habitats in France, Belgium, Sweden and, for the first time by us, Germany. It has also been introduced to the Arabian Peninsula, South Africa, Southeast Asia and Oceania (<https://www.antwiki.org>; (GBIF 2023a); www.gbif.org). Blatrix et al. (2018) already stated that “the actual distribution of this species is poorly understood, as it was only distinguished from *T. albipes* since 2007 (Bolton 2007)”. We found this species mostly amongst plants and Bolton (2007) reported that nests were often found in trees, shrubs, in loose mulch, under debris, in leaf litter, both on and above ground.



Figure 4. *Technomyrmex difficilis* Photo from AntWeb (www.antweb.org); photographer: April Nobile.

The reproduction and caste system of *T. difficilis* is quite peculiar. After the death of the primary queen, ergatoid males and intercastes appear in the nest. The intercastes can make up almost half of the colony (Warner 2003). The ergatoid males and intercastes mate within the colony and budding may occur. Given its high polygyny and polydomony, this species is a very successful disperser, known to form super-colonies and is classified as ‘highly invasive’. This species is also known to feed on and protect sap-sucking hemipterans (Warner 2003).

Technomyrmex vitiensis (Fig. 5)

This species is a 3.5–4.0 mm long, black ant, native to Southeast Asia (Bolton 2007). It has been introduced to California, French Guiana, Suriname, Brazil, the Madagascar region and Oceania. In Europe, *T. vitiensis* was found in greenhouses in the UK, France, the Netherlands, Belgium, Switzerland, Germany, Denmark, Austria, Czech Republic, Poland and Finland (<https://www.antwiki.org>; (GBIF 2023c); www.gbif.org). In fact,



Figure 5. *Technomyrmex vitiensis* Photo from AntWeb (www.antweb.org); photographer: April Nobile.

T. vitiensis is a common greenhouse species in Europe and was found at many greenhouse localities (Pospischil 2011). To our knowledge, this is the first record for Berlin and Brandenburg. *Technomyrmex vitiensis* is part of the *T. albipes* species complex and was only distinguished from *T. albipes* in 2007. In addition, the species of this complex are not easy to distinguish from each other. This also contributes to their poorly known distribution. In general, the biology of *T. vitiensis* is similar to that of *T. difficilis*.

Plagiolepis alluaudi

(Fig. 6)

A very small (1.5 mm) yellow ant native to Africa (Wetterer 2014). The biology of this species has recently been compiled by Blatrix et al. (2018). From Africa, it has been introduced to the Caribbean, California, Asia and Oceania. In Europe, it was found in greenhouses in the UK, Switzerland, Ireland, Germany, Estonia, Poland, the Netherlands, Belgium, Sweden and France (<https://www.antwiki.org>; (GBIF 2023b): <https://www.gbif.org>). To our knowledge, this is the first time this species has been reported from Berlin and Brandenburg. It appears to be a relatively recent invader of greenhouses in Europe. In France, for example, there are no records prior to 1990 (Blatrix et al. 2018). Outdoors, it nests in a variety of very small spaces: under the bark of dead trees, in the holes made by wood-boring insects, under clumps of grass, to name just a few. Indoors, it nests in cavities formed between the stems, petioles or leaves and stems of plants. It prefers to nest above ground, but is often found under flower pots. Blatrix et al. (2018) stated: "The nests are opportunistic, and the ants do not usually build structures or dig burrows. Each nest contains multiple queens, which facilitates their dispersal when materials or plants are exchanged. These ants are in addition very small and pale yellow and can be hard to detect when they are present in small numbers". *Plagiolepis alluaudi* protects and disperses sap-feeding hemipterans and feeds on their honeydew (González-Hernández et al. 1999). This species is also polygynous and polydomous, which contribute to its status as a 'highly invasive' species (<https://www.antwiki.org>).



Figure 6. *Plagiolepis alluaudi* Photo from AntWeb (www.antweb.org); photographer: April Nobile.



Figure 7. Phragmatic soldier of *Cryptotermes cavifrons*: Biosphere Potsdam.

Cryptotermes cavifrons

(Fig. 7)

This dry-wood termite species is native to Florida (Brammer and Scheffrahn 2002). It has been introduced to South Carolina and Nigeria (GBIF 2023d: <https://www.gbif.org>). To our knowledge it has never been recorded for Europe or even Eurasia. *Cryptotermes cavifrons* nests in dry wood and does not need a connection to the ground. We even found a colony in a wooden bench in the greenhouse. Inside the wood, the termites build a nest of interconnected tunnels. From the outside, piles of faecal pellets are the only visible sign of their presence. In *C. cavifrons*, 1–2% of the colony are soldiers, which have phragmatic heads to plug the nest entrances. In dry-wood termites, the workers are replaced by pseudergates. These perform normal worker tasks, but can develop into alates when needed. This species is also unique in that alates are always present in the colony and swarms occur throughout the year (Brammer and Scheffrahn 2002).

Aleptia cf. viatrix

(Fig. 8)

This species of drainflies (*Diptera, Psychodidae*) is expected to be native to the Neotropics as all other species of the genus *Aleptia* occur there (Jaume-Schinkel et al. 2022). Furthermore, the larvae depend on water reservoirs inside bromeliad plants (phytotelmata), which are endemic to the Neotropics. The introduction of this species to other parts of the world seems to be connected to the bromeliad trade (Jaume-Schinkel et al. 2023). *Aleptia viatrix* was only recently described as a new species from the Duque da Terceira botanical garden on the Azores, containing bromeliad plants (Jaume-Schinkel et al. 2022). A following record came from Brisbane in Australia, in an area where bromeliads are cultivated. It then was found in South Africa, whereby only barcodes are available (GBIF 2023e: <https://www.gbif.org>). Our finding of this species was also in a greenhouse part in the Botanical Garden Potsdam, which has lots of bromeliads. We identified this tiny fly by barcoding, which is not that reliable as *Aleptia* is a large



Figure 8. *Aleptia* cf. *viatrix*: Biosphere Potsdam.

genus with 57 described species. From these, barcodes are only available from *A. viatrix* and *A. valentia* on BOLD Systems. However, the barcode has a similarity of 100% with a sample from Brisbane. Thus, this is the first record of this species for continental Eurasia, but further research including morphological analyses are needed.

Geonemertes pelaensis (Fig. 9)

A species of terrestrial nemertines (Hoploneurida, Proserpochmidae), which is probably native to the Indopacific Region (Morffe et al. 2020). It was introduced to the USA, where it has been recorded once from a greenhouse in Denver. It also occurs outdoors in Florida, Central America, the Caribbean, western parts of South America, the Madagascar region and Japan (GBIF 2023f: <https://www.gbif.org>). To our knowledge, this is the first record for Europe. *Geonemertes pelaensis* is a hermaphrodite, a characteristic that makes dispersal much easier (Moore et al. 2001). It extrudes its proboscis when catching prey and/or if stimulated from outside. The diet of *G. pelaensis* is quite enigmatic. On the Seychelles, the species is found to be a predator of small molluscs (Gerlach 1998). On the Ogasawara Islands, however, *G. pelaensis* is a predator of Arthropods, which has led to devastating declines in populations of Isopoda and Amphipoda on that island since the



Figure 9. *Geonemertes pelaensis*: Biosphere Potsdam.

1980s. Shinobe et al. (2017) assume that these differences in diet could be due to different cryptic species involved.

Anisorhynchodemus sp. (Fig. 10)

A species of terrestrial flatworms of which the native range is unknown. The genus has been introduced to Florida, the Caribbean, the Madagascar region, Sri Lanka, Southeast Asia and Oceania (GBIF 2023g: <https://www.gbif.org>). As far as we know, it has never been reported from Germany. The taxonomy within this genus is still under discussion, as incompletely described Rhynchodeminae species are placed in the collective genus *Anisorhynchodemus* (Kawakatsu et al. 2003). Land planaria expert and researcher Leigh Winsor identified this genus on iNaturalist and said: “This appears to be the invasive *Anisorhynchodemus* species found in Florida, the Caribbean and Pacific oceanic islands” (Leigh Winsor pers com.).

The many new records for Berlin, Brandenburg, Germany and even at the continental scale demonstrate how poorly the faunas of greenhouses are known. While most of the tropical species recorded are unlikely to survive in the wild in Germany, they might be transported from our greenhouses to others, potentially reaching suitable climatic conditions along this road. It is not the aim of this study to speculate about the effect of invasive species. However, our findings highlight the immense knowledge gap of the greenhouse invertebrates. Furthermore, this study also shows how many different introduced taxonomic groups can be identified by a combination of the iNaturalist AI, its expert community and barcodes available from BOLD. Both open-access resources enabled the identification of most, but not all, of our species. Obviously, as biodiversity remains imperfectly described, many species complexes are awaiting their taxonomic revisions, making it actually impossible to identify some to the species level.

Indeed, we highly encourage other students, citizen scientists and experts to study greenhouse faunas as well as to publish their records. During our work, we came into contact with several citizen scientists, who told us that they have already seen particular species in greenhou-



Figure 10. *Anisorhynchodemus* sp.: Biosphere Potsdam.

es, but never found it worthwhile to publish these data. Nowadays, it is quite simple to publish single-species records through iNaturalist and other smartphone-based identification tools. To encourage citizen scientists to record greenhouse species, we set up an iNaturalist Project called “Greenhouse fauna of Europe”.

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